

THE INFLUENCE OF TEMPERATURE ON AMPHIBIAN EMBRYOGENESIS

by

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1. INTRODUCTION

In the two preceding papers (24; 25) have been reported some results pertaining to the influence of temperature on the embryonic development of *Amblystoma mexicanum*. It was found that high rearing temperatures caused a considerable loss of reserve material, notably protein (24). When the changes in enzyme content (peptidases) were followed at different temperatures (25), it was found that the maximal amount of enzyme was lower at high temperatures (25 °C).

There is available a considerable amount of literature dealing with the influence of temperature on the embryonic development of amphibia. In the present work several of these papers will be discussed, together with those mentioned above, in an attempt to give a coherent picture of the mechanism of the temperature effect on embryonic development. The discussion will be based upon the effect of temperature on the rate of morphological development, but includes

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also data obtained in the fields of experimental and chemical embryology.

2. INFLUENCE OF TEMPERATURE ON THE RATE OF DEVELOPMENT

In 1914 KROGH published some investigations on the influence of temperature on the rate of embryonic development (21). The observations comprised widely different groups of animals –including amphibia– and they all agreed in showing that, in the temperature range in which normal development takes place, the rate is a linear function of temperature. This observation was, as emphasized by KROGH, in marked contrast to the exponential relationship which is found between temperature and rate of reaction for simple chemical processes. The results found by KROGH did not fit a straight line at all temperatures. At low temperatures the observed values for developmental rate were above the straight line. It was soon after suggested by KANITZ (20) that the straight lines observed by KROGH were the results of superposition of two exponential curves, and that the observed deviations represented the lower part of one such curve. A similar interpretation was advanced by CROZIER (7) who made ARRHENIUS plots (ordinate: logarithm of rate; abscissa: reciprocal of absolute temperature) of KROGH's results. This procedure gave, in most instances, two straight lines intersecting in a sharp break, thus indicating that two reactions with different activation energies (ARRHENIUS Constants) are rate-limiting at low and high temperatures.

The interpretation given by KANITZ and by CROZIER raises the question of the validity of using observed rate-temperature curves to prove the operation of master reactions, *i.e.*, rate-controlling reactions. This question has been discussed by HOAGLAND (14), and some of the arguments he advanced in favor of the above interpretation will be mentioned here. HOAGLAND pointed out that the fit of the ARRHENIUS equation to experimental data is not a test of the presence of a master reaction. Such a fit might equally well be obtained for very complex, summed reactions.

The main support for the idea that certain special master reactions control the rate of most biological phenomena was found in the fact that almost all observed values for the activation energies of biological processes were distributed in rather few groups. This distribution was found statistically significant, and it was furthermore found that each group comprised processes of a special kind.

Thus, biological oxidation processes were found in one group (~ 16000 cal/mol), while the ARRHENIUS Constants for the majority of embryonic growth processes were found in two ranges, viz. 10–13000 cal/mol and 19–23000 cal/mol (NEEDHAM (30)). The two groups observed in the latter instance correspond to the shift observed in embryonic processes, *i.e.* the high values were found at low temperatures, and the low values at high temperatures.

As a support, HOAGLAND also argued that, when shifts were observed, these were most frequently from a value belonging to one group to a value belonging to another group. HOAGLAND emphasized that no specific mechanisms were assumed as to the nature of these shifts, and especially that no kinetic mechanisms had been published because no simple one would give sharp breaks in ARRHENIUS plots.

In biological systems, most of the conditions under which the chemical reactions proceed are beyond experimental control, and it is therefore obvious that the observed values for activation energies, at best, are “apparent activation energies”, and a direct comparison of these values with those obtained from simple, well-defined chemical reactions is, if not unwarranted, at least weighted with a great uncertainty. This point has been emphasized by several authors, and, accordingly, no conclusion or suggestion will, in the present discussion, be based on the absolute values of observed ARRHENIUS constants, although, in some cases, the relative values will be used as supporting evidence.

It might be desirable to have stronger arguments than those produced by HOAGLAND, before the interpretation given by KANITZ and by CROZIER is accepted. Although it is realized that the observed rate-temperature curves are interpretable in other ways, *e.g.*, by changes in the amount of catalyst or by inhibition of the rate-limiting process, rather than by a shift to another rate-controlling process, this latter possibility nevertheless will be adopted in the following discussion because a number of independent experimental results, otherwise difficult to explain, become understandable on this basis.

An interesting observation concerning the influence of temperature on the developmental rate of amphibian embryos was described by BUCHANAN (3) and by RYAN (34). Working with *Amblystoma punctatum* and *Rana pipiens*, respectively, these authors both found that the rate of morphological development at high temperatures could be increased if the embryos were first kept at low temperatures for some time. On the other hand, the development at

low temperatures was retarded by a preceding stay at high temperatures. RYAN thus found that embryos which had been kept for a certain time below 18 °C, when transferred to temperatures above 18 °C, might catch up with, or even get ahead of controls which had remained all the time at the high temperature. Both authors found it necessary to assume two reactions or processes, having different temperature characteristics, to be at work during development.

On the basis of the temperature-rate relationship RYAN suggested that the temperature dependency of the rate-limiting process at high temperatures (II) is linear, in agreement with KROGH. The temperature dependency of the rate-limiting process at low temperatures (I) can, according to RYAN, be described by the ARRHENIUS equation, the activation energy (the ARRHENIUS constant) being 29300 cal/mol (see fig. 1A). This suggestion thus also takes into

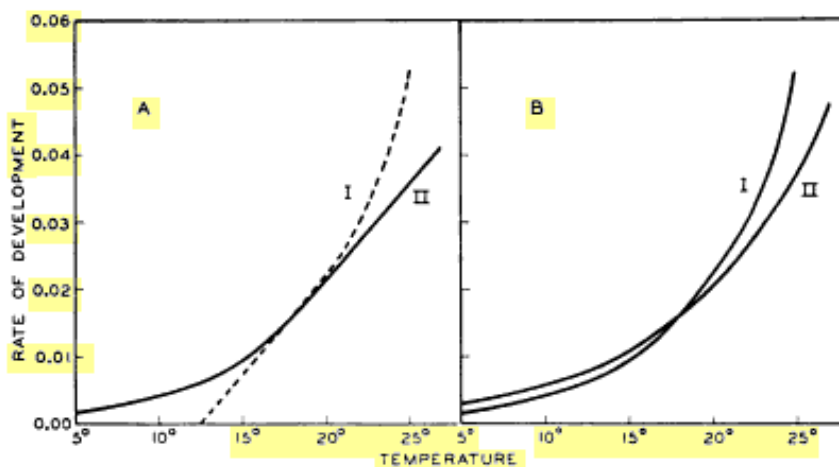


Fig. 1. A. Illustration of RYAN's results. B. Curve I: RYAN's ARRHENIUS curve. Curve II: The second ARRHENIUS curve calculated from RYAN's data. Ordinate: 1000 divided by the time, in minutes, between stages 10 and 20 (RYAN)

account the deviation from the straight line observed by KROGH.

RYAN suggested that a process, once working, would continue for some time after transfer across the transition temperature, and at a rate found by extrapolating to the new temperature (stippled lines, fig. 1A). On the basis of this suggestion, the observed accelerations and retardations could be explained.

However, when a process, which at one temperature is rate-limiting in a succession of reactions, at another temperature ceases to be rate-limiting, then it seems most probable that this process

now may proceed at a greater rate than the new rate-limiting one. Instead of having one curve tangential to the other, one would therefore rather expect the two curves to intersect, as would be the case if mechanism II also followed the *ARRHENIUS* equation, but with a lower activation energy. If we therefore want to retain the idea that two different rate-limiting processes are at work it seems most reasonable to accept the interpretation given by *KANITZ* (20) and by *CROZIER* (7).

From *RYAN*'s data it is possible to estimate an approximate value of 20000 cal/mol for the activation energy of II. The two *ARRHENIUS* curves are shown together in fig. 1 B. From 18 °C to 24 °C the differences between curve II and the straight line are small and could easily be overlooked, but above 24 °C the differences become greater. If the second exponential curve is correct, this difference must indicate that the influence of those factors, which eventually cause the rate to decrease, is becoming measurable.

It will be noticed that *RYAN*'S values do not fit into the two main groups of activation energies for embryonic growth processes, but it should be mentioned, however, that some few embryonic growth processes were found to have *ARRHENIUS* Constants of 27–28000 cal/mol. This fact need not lead to the conclusion that, in some species, different processes are rate-controlling, insofar as the range of the experimental temperature interval may influence the estimated values. Thus, if the lowest temperature is too high, the lowest line will be poorly defined, and, accordingly, often neglected, whereas the values at high temperatures, just before the maximum developmental rate is reached, if included, may give another, approximately straight line with a very small slope, corresponding to a low activation energy. This point will not be further discussed here because the absolute values of the activation energies are unimportant for the present treatment of the problem.

It was mentioned above that *RYAN* (34), in order to explain the observed accelerations and retardations of developmental rate, suggested that a process, once at work, continues for some time after transfer across the transition temperature. If it be accepted that the observed developmental rate-temperature curves are composed of two *ARRHENIUS* curves, it is seen that the explanation given by *RYAN* can no longer account for the observed accelerations and retardations. According to *RYAN* one should, in this case, rather expect accelerations irrespective of the direction of transfer. It is therefore necessary to attempt to explain the observed changes in developmental rate in another way.

3. THE RATE-LIMITING PROCESSES

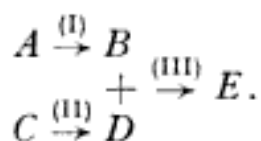
It was mentioned in the preceding section that the present discussion is based on the assumption that the change in activation energy

observed for the embryonic development in amphibia represents a change from one rate-controlling process to another, although it is realized that other possibilities of interpretation exist.

Before entering on a discussion of the experimental data which suggest that two rate-limiting reactions actually are at work, a simple mechanism will be suggested which, besides being in agreement with the experimental results, also gives a sharp break in an ARRHENIUS plot (cf. the requirement emphasized by HOAGLAND, mentioned in the preceding section).

Let us assume that two kinds of substances, B and D , must be supplied, in a given ratio, before morphological development can proceed. Let us furthermore assume that B and D are the products of two processes, I and II, respectively, which are mutually independent, and also independent of the process $B + D \rightarrow E$ (III), where E represents the morphological structures by which the overall process is measured.

This suggested mechanism is illustrated by the following scheme:



It will also be assumed that the rate of III is controlled by the rate of supply of either B or D , *i.e.*, the potential rate of III is great compared with the rates of I and II. If B and D are supplied in the ratio needed for III, no accumulation will occur, but if one of these substances is supplied in excess, accumulation must take place. If the accumulated substances are, or are transformed into, low-molecular compounds, losses by diffusion may occur.

If I and II, or rather the controlling steps in each of these processes, have different activation energies, and if their rates at some intermediate temperature are such that B and D are supplied in the correct ratio, we can conclude from the assumptions stated above that at low temperatures the process with the highest activation energy, say I, will control the rate of morphological development, while the products of II, D , will be accumulated. Conversely, at high temperatures II will be rate-limiting, while B will be accumulated.

If the rate of morphological development is determined according to this mechanism, one will obtain a break when the data are plotted according to ARRHENIUS, because the two rate-limiting processes are independent.

The overall process with which we are dealing must consist of a great number of single reactions. How these are distributed among the three separate processes considered (I, II, and III), cannot be said, but the assumed independence of I and II suggests that these occur early in the overall sequence, *i.e.* that most of the single reactions must be located in III.

After these general remarks we will turn to the experiments dealing with the influence of temperature on amphibian embryogenesis, in an attempt to get some idea about the nature of the rate-controlling processes.

3.1. The possible nature of the rate-limiting process at low temperatures.

According to the mechanism suggested above it is clear that one of the rate-limiting processes may be mobilization of reserve material. This process must be an early one in the overall sequence and may be expected to be independent of other processes. If mobilization consists of hydrolytic reactions, accumulation of reaction products should not have much influence on its rate, since the equilibrium of hydrolytic processes is reached only after almost complete hydrolysis. This argument is not valid if special mechanisms for resynthesis of inactive reserve material are present, but this possibility seems unlikely.

Let us therefore assume that I represents the mobilization of reserve material. This means that the rate of development at low temperatures is controlled by the rate of supply of reserve material, as suggested by TEN CATE (36). At higher temperatures mobilized reserve material should be accumulated in the embryos, and, since the mobilization presumably consists in a production of low-molecular, diffusible compounds, these might be lost. Such loss was actually observed in (24), demonstrating the independence and the high temperature coefficient (activation energy) of the mobilization of reserve material. This, of course, does not prove conclusively that the suggested mechanism is correct.

The results of enzyme determinations at different temperatures also support the suggestion. At low temperatures the low rate of supply of reserve material may account for the lower content of cholinesterase (TEN CATE, KOOIJ, and ZUIDWEG (37)), whereas the reduced content of peptidases at high temperatures was accounted for by the loss of reserve protein (25).

In the suggested mechanism we are dealing with the morphological development, the rate of which was shown in a preceding paper (24) not to be limited by the rate of energy supply. Process I must therefore represent the mobilization of synthetic reserves (notably protein), but there are some results which suggest that the rate of supply of energy sources (notably fat) runs parallel to the rate of protein supply. These results will be discussed briefly in the following paragraphs.

It was shown in a preceding paper (24), that the amounts of reserve material (fat and protein) left at the time of the respiratory maximum were quite different at different temperatures. The lower the rearing temperature, the more reserve material was left at this time.

The respiratory maxima occurred a short time after the onset of protein combustion (24), and at a time when, it could reasonably be assumed, little or no inactive reserve protein was left. Under these circumstances consumption of protein must lead to decreases in the amounts of active proteins (enzymes *etc.*, cf. (25)), which again may cause a decrease in the rate at which reserve material is utilized, and accordingly, at which oxygen is consumed.

It was suggested, but not discussed in detail, that the rate at which fat is supplied for combustion may play a rôle in deciding when the respiratory maximum occurs (24). Here an attempt will be made to explain how this may come about, based upon the two possibilities which were advanced to account for the onset of protein combustion (24).

According to the first one, protein is mobilized when the rate of oxygen consumption is limited by the rate at which fat is supplied. In this case an earlier onset of protein combustion may cause an earlier occurrence of the respiratory maximum. According to the second possibility protein combustion must begin when a certain developmental stage is attained. It was shown in (24) that the rate of oxygen uptake –and hence the total oxygen uptake– does not limit the rate of morphological development. Therefore, the critical stage may be reached even if the rate of oxygen uptake has been limited by the rate of fat supply.

In both cases one might expect to find the total oxygen consumption up to the time of the respiratory maximum ($\Delta O_{2(M)}$) to be lower when the rate of fat supply is limiting than under conditions where this is not the case. Now according to the ideas of the present discussion, the rate of supply of reserve material is a limiting factor at low temperatures. One might therefore expect $\Delta O_{2(M)}$ to increase with the temperature. At high temperatures, however, a loss of reserve material occurs which causes a decrease in both $\Delta O_{2(M)}$ and in the total oxygen consumption until the time of exhaustion, even though the difference between those two quantities is greatly reduced (24).

These considerations lead us to expect $\Delta O_{2(M)}$ to attain a maximum value at some intermediate temperature. This specific question has not yet been investigated, but TEN CATE (36) has found that at the end of the external morphological development the oxygen uptake in *Rana fusca* was higher at 15.5 °C than at 20 °C and 12 °C. This result indicates agreement between ex-

pected and observed results but they cannot be regarded as conclusive; the endpoint of TEN CATE'S measurements is not sufficiently well-defined for the present purpose.

It is possible, on the basis of the idea that the rate of mobilization of reserve material is rate-limiting at low temperatures but proceeds too fast at high temperatures, to give a plausible explanation of the retardation in developmental rate occurring after transfer from a high to a low temperature. If embryos are kept for some time at a high temperature, they will lose more of their reserve material in reaching a certain stage than do the controls at low temperature. This loss may cause the retardation in morphological development observed when the embryos are transferred to conditions, under which the rate of supply of reserve material is the rate-limiting process.

3.2. The rate-limiting process at high temperatures.

3.21. The acceleration of developmental rate.

To explain the accelerations observed by BUCHANAN (3) and by RYAN (34), it was suggested by BUCHANAN (4) that accumulation of rate-limiting "substance" occurs when embryos are kept at low temperatures. This means, according to the suggested mechanism, that process II proceeds relatively faster than I, and that, consequently, the product of II (D) is accumulated.

If reaction II, as assumed, is completely independent of and has a lower temperature coefficient than I, there is no difficulty in accepting this possibility. However, I was suggested to be the mobilization of reserve material, and it is difficult to imagine a process, at least a synthetic one, which is completely independent of supply of reserve material.

It is of course possible that the substances used in II (C) come from another source, different from the reserve material A , but it is more likely that C , to some extent at least, is mobilized reserve material, B . In this case, however, II is not independent of I, and no accumulation of D should therefore occur. It should rather be expected that the available amount of B would be evenly distributed between II and III. We therefore will have to attribute to II special qualities which will make possible such an accumulation.

We shall have to conclude that II, in competition for reserve material, available only in limited quantities, is favored relative to other processes which need these substances to proceed. This may

occur if the reaction in question is irreversible, the resulting substances being "stable", while the other reactions are reversible, leading to "labile" substances.

This explanation may not be correct; in the use of the word "irreversible" in the following discussion this particular mechanism will not be implied. The word will imply only that a reaction may proceed with relatively little change in rate under conditions, which reduce the rates of other reactions. Besides being "irreversible" in this special sense, it is necessary also to maintain the requirement that this process is an early step in the overall process. It is clear that if an "irreversible" process is located at the end of the total process, competition is excluded because all the unfavored reactions have occurred, and no possibility of accumulation exists. To denote the quality that the process must be an early step the expression "independent" will be used, for lack of a better one.

The plausibility of the suggestion that the rate-limiting process (II) may go ahead of the morphological development at low temperatures is supported by the fact that certain processes actually do so. It was found by TWITTY (38) that at low temperatures the determination of the direction of the ciliary beat of the epidermis was achieved at a much earlier morphological stage than at room temperature (*Amblystoma punctatum*). Similarly, TEN CATE (36) has shown that if the eye primordium be removed in the neural plate stage (stage 14) of *Rana esculenta* embryos kept at 10 °C and 25 °C, respectively, lenses would self-differentiate in all cases in the low temperature embryos, while less than 10 per cent of the animals at high temperature showed lens formation. In mentioning these results it is of course not implied that the determination process is the rate-limiting process discussed in this section, but these processes may well be interrelated.

If we grant the "irreversibility" and the "independence" of the second rate-limiting mechanism (II), it is possible to explain why embryos kept at low temperatures develop at greater rates than the controls, on transfer to higher temperatures. After the transfer reaction II will become rate-limiting, but the accumulated products of II (*D*) will permit the morphological development to proceed at a rate higher than that dictated by reaction II, until all *D* has been consumed.

Unfortunately, this does not explain how the embryos get ahead of the controls, because after all, it seems most likely that, at the time

of transfer, there has been produced more substance by reaction II in the controls than in the low temperature embryos.

It is possible to make some suggestions to explain this phenomenon, based on the fact that the developmental rate (measured between stages 10 and 20 (RYAN (34))) follows curve II only in a very narrow temperature interval. It seems reasonable to assume that the straight line which represents the temperature relationship at the highest temperatures before the maximum still shows the rate of mechanism II, rather than of a third rate-limiting process. In that case obviously something happens at high temperatures which reduces the rate of process II to values lower than predicted from the rate temperature curve. This may be the loss of some specific substrates for process II (cf. (24)) which may cause its rate to decrease.

Under these conditions the low temperature embryos would, after the transfer, have the advantage of possessing more synthetic reserves and of containing more of the products of reaction II (*D*) than the controls in the same stage of development. The latter fact may be important if among the products of reaction II are accumulated more of the enzymes responsible for II, as seems most reasonable. It seems probable that the embryo, at least for some time after the transfer, might be able to synthesize *D* at a rate corresponding approximately to curve II, rather than to the straight line of morphological development (KROGH (21), Ryan (34)). Later the retarding effects of high temperature must become effective, and the developmental rate decrease. Measuring the time taken between two relatively widely separated stages, as RYAN did, one should not be able to detect this phenomenon. In BUCHANAN'S paper (3) where the time-stage curves for controls and cold-treated embryos are shown, it is seen that the greatest acceleration occurs immediately after the transfer.

The basic idea in the suggestion above is that when normal development is inhibited, by lack of synthetic reserves, as at low temperatures in the present instance, then the normal rate-limiting process continues more or less unaffected, thus getting ahead of the other developmental processes. Therefore, when the inhibiting factor is eliminated, the development will proceed at a rate higher than normal, until it has caught up with the lead of the rate-limiting process. This phenomenon has often been observed on various objects (mammals), *e.g.* in some of the classical experiments of OSBORNE and MENDEL on rats. Although the experimental animals were not amphibia, a fact which of course dictates caution when comparison is made, the results will nevertheless be summarized briefly here, to demonstrate the analogy.

Growing young rats were fed protein-deficient food (OSBORNE and Mendel (32), JACKSON (18)), or were underfed (JACKSON (19)), the amounts of food being adjusted so as to keep the body weight constant for several weeks. When afterwards food was given *ad libitum* the rats began to gain weight at a considerably higher rate than the controls, reaching approximately the same final weight as the latter. Here also it seems most reasonable to assume that the rate-limiting processes have continued at the cost of other processes.

The results of LIMSON and JACKSON (23) supply direct evidence that the increase of parts of the body can go on at the expense of other parts. These authors compared the size or weight of various organs and tissues before and after the starvation periods. They found that some increased, some remained constant, and some decreased. Some few examples may serve to illustrate this. The skeletal length, *i.e.* the body and tail length increased. The weight of the liver also increased, whereas, *e.g.*, kidney and brain remained constant. A decrease was found in the weight of muscles and integument.

3.22. The possible nature of the process.

It was concluded in the preceding section that the rate-controlling reaction at high temperatures must be characterized by being “irreversible”, *i. e.* by being able, under certain circumstances, to proceed uninhibited, even if the rates of other reactions are reduced, and by being “independent”, *i.e.* being an early step in the overall process. Besides this we also know about this reaction that it must have a relatively low activation energy (temperature coefficient).

At our present state of knowledge it is impossible to state definitely the nature of this second rate-limiting reaction. However, as will appear from the following discussion, there is much evidence in favor of the suggestion that this rate-controlling reaction is cell division, or rather some unknown step in the process of cell division. It can be shown by various experimental results that cell division, or processes related to it, occupy a unique position among biological processes by having exactly those qualities mentioned above.

With respect to the “independence” of the process it is known that in embryonic development most cells have stopped dividing at the time the cells begin to undergo differentiation and growth, *i.e.* that mitosis precedes these other developmental processes. This fact is illustrated very clearly on a quantitative basis by comparing the curves which represent the changes in various substances during development, *e.g.* nucleic acids, enzymes, etc. (LØVTRUP (26)). It is then seen that DNA (measured as desoxyribonucleosides (HOFF-

JØRGENSEN (15)) is the first substance among many to increase in amount, indicating the formation of new nuclei, *i.e.* new cells. This fact is the more remarkable since the greatest relative increase in cell number, during segmentation, occurs without any increase in DNA (HOFF-JØRGENSEN and ZEUTHEN (16); ZEUTHEN (39)).

There are various results to be found in the literature which indicate that mitosis in general has a rather low temperature coefficient (cf. NEEDHAM (31)). It is of special interest for the present discussion that all observations on the influence of temperature on amphibian development have shown that the temperature coefficient for the early stages, which are measured by mitoses, is lower than the temperature coefficient for the later development (KROGH (21), RYAN (34; 35), cf. also NEEDHAM (30)). RYAN thus estimated that the activation energy for this part of the development is about 20000 cal/mol in *Rana pipiens*. This value agrees encouragingly with the value estimated for the activation energy of the second rate-limiting reaction in the same species.

With respect to the “irreversibility” of the reaction there are several experimental results which, directly or indirectly, suggest that the process of cell formation has this quality.

It is a general experience, that larvae reared at high temperatures are smaller than larvae reared at low temperatures. DOMS (12) could find only slight morphological differences (*e.g.* size of gills) between larvae reared at different temperatures; it must therefore be concluded that the final morphological stage is unaffected by temperature. CHAMBERS (6) was able to show that the difference in size is due to the smaller size of the individual cells of the high temperature animals in *Rana temporaria* and *Rana esculenta*, and this result was later confirmed by DOMS (12)). This observation is in perfect agreement with the suggestion that the mitotic rate, after depletion of the DNA “store”, is only slightly influenced by the available reserve material, while the amount of cytoplasm (as indicated by the amount of peptidases (25)) is reduced because of the great loss of protein which occurs at high temperatures.

The constancy of the number of cells, *i.e.* the irreversibility of the process of cell formation, has been investigated by MORGULIS (29), who found, in the salamander *Diemyctylus viridescens*, that the decrease in cell size caused by starvation could amply account for the total loss of weight, from which he inferred that the cell number remained constant.

An interesting finding which demonstrates the priority of the cell division under conditions of starvation, has been shown by BRUES, DRURY and BRUES (2), who investigated the regeneration of rat liver after partial hepatectomy. They found that starvation had a great influence on the increase in mass, whereas it had no influence whatever on the increase in cell number.

It should be stressed that the “irreversibility” attributed to the process of cell division does not exclude the possibility that cells, under certain circumstances, may degenerate and die.

The amount of *DNA* is known to be doubled before each cell division (BOIVIN, VENDRELY and VENDRELY (1)). This quantity is therefore proportional to the number of cells present, and can be used to follow changes in this latter quantity (DAVIDSON and LESLIE (9)).

The works of DAVIDSON (8) and of DAVIDSON and MCINDOE (11) have clearly shown that starvation, although causing losses of a number of other cell constituents, has no effect at all on the amount of DNA in rat liver, nor on the amount in isolated nuclei, thus confirming MORGULIS' inference. Likewise, MANDEL, JACOB and MANDEL (27) have shown that in rats kept for a long time on a protein-free diet, no changes occur in the amount of *DNA* in liver, kidney and brain. CAMPBELL and KOSTERLITZ (5) found that liver *DNA* was unaffected by variations in dietary protein.

The results above have been confirmed by some experiments on planarian worms, which are known to be able to endure very prolonged starvation. The animals were starved for more than two months, during which time their total nitrogen content decreased to about 10 per cent, while *DNA* was found to remain practically constant (HOFF-JØRGENSEN, LØVTRUP and LØVTRUP (17)). All these results do indeed place *DNA* in a remarkable position among the cell constituents, and indicate the irreversibility of cell division.

This physiological stability of *DNA* might expectedly also be reflected in a very low rate of turnover, as measured by isotopes. DAVIDSON and LESLIE (10) discussed this question, and found that the available experimental evidence (from P^{32} and N^{15} studies) suggested that *DNA* might not participate in the dynamic equilibrium of the body constituents. This was also found by MARSHAK (28) who showed that the rate of incorporation of P^{32} in *DNA* was very low in non-growing tissues, but not in growing tissues.

It is outside the scope of the present paper to give a detailed discussion of this question, but some few recent experiments should

be mentioned which demonstrate a relatively high rate of turnover of *DNA*. After feeding glycine (LE PAGE and HEIDELBERGER (22)) or glycine and serine (ELWYN and SPRINSON (13)) labelled with C^{14} it was found that the specific activities of the purines in DNA and RNA of rat liver were of the same order. REICHARD and ESTBORN (33) found by feeding N^{15} -labelled desoxyribosides that they were taken up in the DNA fraction of rat liver. These results thus indicate that a certain turnover occurs. It is beyond the scope of the present work to attempt to reconcile these two apparently contradictory groups of results. For the present discussion it must suffice to say that the isotope experiments to some extent confirm the unique position of DNA, as shown by the starvation experiments.

4. CONCLUSION AND SUMMARY

Various observations concerning the influence of temperature on amphibian embryogenesis have repeatedly been interpreted as showing that two different reactions control the rate of development at high and low temperatures. Until now the nature of these two reactions has remained completely obscure, except for rather unsubstantiated guesses. The present paper is an attempt, based on the available experimental evidence, to narrow down the possibilities with respect to the nature of these reactions. As a result the following hypothesis has been advanced: The rate of morphological development at low temperatures is controlled by the rate at which reserve material is mobilized, and at high temperatures by the rate at which cell division proceeds. Further experimentation is needed before this hypothesis can be considered proved. However, even if the hypothesis is correct, it still remains to be shown which particular steps in the mobilization of reserve material and in cell division are the rate-controlling ones.

Many attempts have been made by experimental embryologists to dissociate some of the complex processes going on during embryonic development. By such dissociations it may be expected to gain some information both about the separate processes, and about the interrelations between them (cf. NEEDHAM (31)). Some of the most interesting results of this kind have been obtained by keeping the embryos at extreme temperatures. These results have been mentioned and discussed in the present and the two preceding papers (24; 25), and may be summarized in the following way: At low temperatures and in the beginning of development,

certain processes will go ahead of morphological development. These processes include determination and, possibly, the one controlling the rate of development at high temperatures (cell division).

At low temperatures and at the end of prefeeding development, it has been found that the total oxygen consumption and the content of enzymes are lower than at intermediate temperatures.

At high temperatures and in the beginning of development, a considerable loss of reserve material has been observed. The total oxygen consumption up to a given stage is somewhat reduced, but the rate of morphological development is apparently not affected.

At high temperatures and at the end of prefeeding development, the amounts of enzymes and the total oxygen consumption are reduced, but, in spite of this, the morphological development is apparently carried to the normal endpoint. All these dissociations are accounted for by the hypothesis suggested above.

It seems possible, on the basis of the results discussed in the present and in the two preceding papers, to classify some of the processes which go on during embryogenesis according to the extent to which they are effected by unfavorable conditions:

Certain processes, such as determination and cell division, go on more or less unaffected even by conditions under which the rate of morphological development is considerably reduced (by reduction in the rate of supply of reserve material).

The rate of morphological development may be reduced by a fall in the rate of supply of reserve material, but it is not influenced by reduction of the total amount of energy sources or synthetic reserves available.

The maximum amounts of enzymes per embryo are influenced by the total amount of reserve material.

REFERENCES

1. BOIVIN, A., R. VENDRELY et C. VENDRELY: Biochimie de l'hérédité. L'acide desoxyribonucleique du noyau cellulaire, dépositaire des caractères héréditaires; arguments d'ordre analytique. *Compt. rend. Acad. Sci.* **226**, 1061–1063 (1948).
2. BRUES, A. M., D. R. DRURY and M. C. BRUES: A quantitative study of cell growth in regenerating liver. *Arch. Path.* **22**, 658–673 (1936).
3. BUCHANAN, J.W.: Developmental acceleration following inhibition. *J. Exp. Zool.* **79**, 109–127 (1936).

4. BUCHANAN, J.W.: Developmental rate and alternating temperatures. J. Exp. Zool. **83**, 235–248 (1940).
5. CAMPBELL, R. M. and H. W. KOSTERLITZ: The effects of growth and sex on the composition of the liver cells of the rat. J. Endocrinol. **6**, 308–318 (1950).
6. CHAMBERS, R.: Einfluss der Eigrösse und der Temperatur auf das Wachstum und die Grösse des Frosches und dessen Zellen. Arch. mikrosk. Anat. **72**, 607–661 (1908).
7. CROZIER, W. J.: On curves of growth, especially in relation to temperature. J. Gen. Physiol. **10**, 53–73 (1926).
8. DAVIDSON, J. N.: Some factors influencing the nucleic acid content of cells and tissues. Symp. Quant. Biol. **12**, 50–59 (1947).
9. DAVIDSON, J. N. and I. LESLIE: A new approach in the biochemistry of growth and development. Nature, **165**, 49–53 (1950).
10. DAVIDSON, J. N. and I. LESLIE: Nucleic acids in relation to tissue growth: A review. Cancer Res. **10**, 587–594 (1950).
11. DAVIDSON, J. N. and W. M. MCINDOE: Phosphorus compounds in the cell nucleus. Biochem. J. **45**, XVI (1949).
12. DOMS, H.: Über den Einfluss der Temperatur auf Wachstum und Differenzierung der Organe während der Entwicklung von *Rana esculenta*. Arch. mikroskop. Anat. **87**, 60–95 (1915).
13. ELWYN, D. and D. B. SPRINSON: The extensive synthesis of the methyl group of thymine in the adult rat. J. Am. Chem. Soc. **72**, 3317–3318 (1950).
14. HOAGLAND, H.: “Master reactions” and temperature characteristics. J. Cell. Comp. Physiol. **10**, 29–36 (1937).
15. HOFF-JØRGENSEN, E.: A microbiological assay of deoxyribonucleosides and deoxyribonucleic acid. Biochem. J. **50**, 400–403 (1951).
16. HOFF-JØRGENSEN, E. and E. ZEUTHEN: Evidence of cytoplasmic deoxyribosides in the frog’s egg. Nature, **169**, 245–246 (1952).
17. HOFF-JØRGENSEN, E., E. LØVTRUP and S. LØVTRUP: Changes in deoxyribonucleic acid and total nitrogen in planarian worms during starvation (in preparation).
18. JACKSON, C. M.: Recovery in rats upon refeeding after prolonged suppression of growth by dietary deficiency of protein. Am. J. Anat. **58**, 179–193 (1936).
19. JACKSON, C. M.: Recovery of rats upon refeeding after prolonged suppression of growth by underfeeding. Anat. Rec. **68**, 371–381 (1937).
20. KANITZ, A.: Temperatur und Lebensvorgänge. Berlin (1915).
21. KROGH, A.: On the influence of the temperature on the rate of embryonic development. Z. allg. Physiol. **16**, 163–177 (1914).
22. LE PAGE, G. A. and C. HEIDELBERGER: Incorporation of glycine-2-¹⁴C into proteins and nucleic acids of the rat. J. Biol. Chem. **188**, 593–602 (1951).

23. LIMSON, M. and C. M. JACKSON: Changes in the weights of various organs and systems of young rats maintained on a low-protein diet. *J. Nutrition* **5**, 163–174 (1932).
24. LØVTRUP, S.: Utilization of reserve material during amphibian embryogenesis at different temperatures. *Compt. rend. Lab. Carlsberg. Sér. chim.* **28**, 400–425 (1953).
25. LØVTRUP, S.: Changes in the content of peptidases during amphibian embryogenesis at different temperatures. *Compt. rend. Lab. Carlsberg, Sér. chim.* **28**, 426–443 (1953).
26. LØVTRUP, S.: Chemical differentiation during amphibian embryogenesis. *Compt. rend. Lab. Carlsberg, Sér. chim.* (in preparation).
27. MANDEL, P., M. JACOB et L. MANDEL: Étude sur le métabolisme des acides nucléiques. I. Action du jeûne protéique prolongé sur les deux acides nucléiques du foie, du rein et du cerveau. *Bull. Soc. Chim. Biol.* **32**, 80–88 (1950).
28. MARSHAK, A.: Evidence for a nuclear precursor of ribo- and desoxyribo- nucleic acid. *J. Cell. Comp. Physiol.* **32**, 387–406 (1948).
29. MORGULIS, S.: Studies of inanition in its bearing upon the problem of growth. I. *Arch. Entwickl. Mech.* **32**, 169–268 (1911).
30. NEEDHAM, J.: *Chemical embryology*. Cambridge (1931).
31. NEEDHAM, J.: *Biochemistry and morphogenesis*. Cambridge (1942).
32. OSBORNE, T. B. and L. B. MENDEL: Acceleration of growth after retardation. *Am. J. Physiol.* **40**, 16–20 (1916).
33. REICHARD, P. and B. ESTBORN: Utilization of desoxyribosides in the synthesis of polynucleotides. *J. Biol. Chem.* **188**, 839–846 (1951).
34. RYAN, F. J.: Temperature change and the subsequent rate of development. *J. Exp. Zool.* **88**, 25–54 (1941).
35. RYAN, F. J.: The time-temperature relation of different stages of development. *Biol. Bull.* **81**, 432–440 (1941).
36. TEN CATE, G.: Experiments on amphibian eggs (Biochemistry of the embryo) in M. N. WOERDMAN and CHR. P. RAVEN: *Experimental Embryology*. New York, Amsterdam (1946).
37. TEN CATE, G., J. S. KOOIJ and M. H. J. ZUIDWEG: The effect of temperature on the synthesis of the enzyme cholinesterase in amphibian embryos. *Konink. Nederl. Akad. Wetensch., Proc. Ser. C.* **54**, 157–170 (1951).
38. TWITTY, V. C.: Experimental studies on the ciliary action of amphibian embryos. *J. Exp. Zool.* **50**, 319–344 (1928).
39. ZEUTHEN, E.: Segmentation, nuclear growth and cytoplasmic storage in eggs of echinoderms and amphibia. *Pubb. Staz. Zool. Napoli*, **23**, Suppl. 47–69 (1951).